

## **EXHIBIT B**

UNITED STATES DISTRICT COURT

DISTRICT OF MASSACHUSETTS

IN RE: COLUMBIA UNIVERSITY  
PATENT LITIGATION

MDL NO. 1592

IMMUNEX CORPORATION, a Washington  
Corporation and AMGEN INC., a Delaware  
Corporation,

Plaintiffs,

vs.

THE TRUSTEES OF COLUMBIA  
UNIVERSITY in the City of New York,  
a New York Corporation,

Defendant.

Civil Action No. 04-10740-MLW

C.D. Cal. No. CV 03-4349 MRP (CWx)

AND RELATED COUNTERCLAIM

**EXPERT REPORT OF BRUCE J. DOLNICK**

would be stably incorporated in the chromosomal DNA of the transformed cells. In addition, the insertion of DNA I and a selectable marker into plasmid or phage vectors for the purpose of generating large amounts of DNA for transformation or other experiments was a common procedure. *E.g.*, Weissmann, "Future Trends: Reversed genetics," *TIBS* N109-N111, N110 (1978); Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248 (1/1980); *see also* my discussion in Section 3(e)(i)(2)(a), below. This close linking of DNA I and DNA II would have increased the expectation that the process would result in the stable incorporation of DNA I as well as DNA II. *See, e.g.*, "Stable Incorporation of DNA II is Obvious from Culturing Cells Under Suitable Conditions," Appendix B.

29. By February 25, 1980, it was known that selection after cell transformation allowed one to identify and isolate cells that contained stably incorporated DNA encoding a selectable phenotype and a linked gene encoding a protein of interest. Examples include: Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248, 247 (1980); Willecke *et al.*, "Cotransfer of Two Linked Human Genes into Cultured Mouse Cells," *Proc. Natl. Acad. Sci. U.S.A.* 73(4):1274-1278, 1277-1278 (1976) ("Under selective conditions all gene transfer cells in which the donor genes are stably integrated into a recipient chromosome should overgrow those cells which harbor non-integrated donor chromosomal fragments. Thus the stable phenotype is more likely to be expressed the longer the selection pressure is applied during growth of gene transfer cells."); Willecke, "Results and Prospects of Chromosomal Gene Transfer between Cultured Mammalian Cells," *Theor. Appl. Genet.* 52, 97-104 (1978).

30. In addition, in 1978, Pellicer *et al.*, reported the stable integration of a *tk* gene into the DNA of all transformants and stated: "Biochemical transformation involves the stable acquisition of a new trait which can be readily detected by the ability of cells to grow under appropriate selective conditions. . . . [A]ll transformed clones examined contain . . . the *tk* gene [] covalently integrated into the DNA of all transformants . . . . These results demonstrate the stable integration of HSV DNA into mammalian cells." Pellicer *et al.*, "The Transfer and Stable Integration of the HSV Thymidine Kinase Gene into Mouse Cells," *Cell* 14:133-141, 133 (1978); *see also* Wigler *et al.*, "Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total Cellular DNA as Donor," *Cell* 14:725-731, 725, 729 (1978); Scangos *et al.*, "Gene Amplification as a Concomitant to Chromosome Mediated Gene Transfer," in *Eucaryotic Gene Regulation* (Axel *et al.* eds.) Academic Press, Inc., pg. 445-456, 445 (1979) ("Recipient cell lines initially express the transferred gene unstably (lose the gene at a characteristic rate in nonselective medium) and convert to stable expression upon prolonged cultivation.").

31. One of ordinary skill in the art prior to February 25, 1980 would have expected amplified genes identified under selective conditions to be stably incorporated into chromosomal DNA. In my personal experience, cells containing amplified *dhfr* genes and isolated under selective conditions always had the amplified *dhfr* genes stably incorporated into chromosomal DNA. For example, in Dolnick *et al.*, "Correlation of Dihydrofolate Reductase Elevation with Gene Amplification in a Homogeneously Staining Chromosomal Region in L5178Y Cells," *J. Cell Bio.* 83:394-402 (1979), I and my co-authors reported a MTX-resistant murine cell line wherein the *dhfr* gene was amplified and stably incorporated in the chromosomal DNA. *See also* Schimke *et al.*, "Gene Amplification and Drug Resistance in Cultured Murine Cells," *Science* 202:1051-

1055, 1054 (1978) ("We have recently shown that a similar homogenously staining region on a single chromosome of a MTX-resistant Chinese hamster ovary cell line contains the DHFR genes by in situ hybridization with mouse DHFR cDNA (25). Thus at least in the Chinese hamster ovary cell line, the genes, which are stably amplified, are located to a chromosome."). By February 1980, therefore, it would have been obvious to a person of ordinary skill in the art to stably incorporate the DNA II in the transformed cell of claim 55 into the chromosomal DNA of the cell.

32. In addition, those of ordinary skill in the art would have known that it is preferable to study a cell line containing DNA I and DNA II stably incorporated because any data resulting from the study of such cells would be more reproducible. Stable incorporation would have been a desired result that those of ordinary skill in the art often tested for and it was a common outcome. By February 1980, it was therefore obvious to a person of ordinary skill in the art to stably incorporate DNA I and/or DNA II in the transformed cell of claim 55 into the chromosomal DNA of that cell.

33. Therefore, it would have been obvious to one of ordinary skill in the art as of February 25, 1980 that the construct in claim 55 is stably incorporated.

**c) The "construct" effective for producing the proteinaceous material in claim 1 is obvious from claim 55**

34. I understand the term "the construct being effective for producing the proteinaceous material" in claim 1 of the '275 patent to mean that the DNA I in the "construct" is capable of being expressed when it is introduced into the eucaryotic cell. It would have been obvious to one of ordinary skill in the art as of February 25, 1980 that the "foreign DNA I [that] codes for proteinaceous material," in claim 55 would be "effective for producing the proteinaceous material" for which it codes.

35. It would have been obvious to one of ordinary skill in the art as of February 25, 1980 that the cells made in the process of claim 55/54 would be effective for producing the protein encoded by DNA I. By February 25, 1980, various groups reported or suggested that a DNA I was effective for producing, and/or actually produced the proteinaceous material after transformation with a selectable marker and culturing under suitable conditions. Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248, 247 (1/1980); Willecke, *et al.*, "Cotransfer of Two Linked Human Genes into Cultured Mouse Cells," *Proc. Natl. Acad. Sci. U.S.A.* 73(4):1274-1278, 1274-1275 (1976); Miller and Ruddle, "Co-transfer of Human X-linked Markers into Murine Somatic Cells via Isolated Metaphase Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 75(7):3346-3350, 3346, 3348 (1978); Weissmann *et al.*, "Expression of cloned viral and chromosomal plasmid-linked DNA in cognate host cells," in *From Gene to Protein: Information Transfer in Normal and Abnormal Cells*, Proceedings of the Miami Winter Symposia (held 1/1979) (Russell et al. eds.) Academic Press, Inc., pgs. 99-132 (1979); Mantei *et al.*, "Rabbit  $\beta$ -globin mRNA production in mouse L cells transformed with cloned rabbit  $\beta$ -globin chromosomal DNA," *Nature* 281(5726):40-46, 40, 42 (1979); Weissmann, "Future Trends: Reversed Genetics," *TIBS* N109-N111, N110 (1978). See, e.g., "Proteins Are Produced in the Transformed Cells Cultured Under Suitable Conditions," Appendix B.

which would also contain a DNA I encoding a protein. See, e.g., Weissmann, "Future Trends: Reversed genetics," *TIBS* N109-N111, N110 (1978); Weissmann *et al.*, "Expression of cloned viral and chromosomal plasmid-linked DNA in cognate host cells," in *From Gene to Protein: Information Transfer in Normal and Abnormal Cells* (Russell *et al.* eds.) Academic Press, Inc., pgs. 99-132, 99 (1979). Chang *et al.* suggested the use of a cloned *dhfr* gene as a selectable marker in a eukaryotic cloning vector. Chang *et al.*, "Phenotypic Expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase," *Nature* 275:617-624, 623 (1978). Therefore, one of ordinary skill in the art as of February 25, 1980 would have thought that the use of a cloning vector containing DNA I linked to a DNA II to transform cells was obvious.

65. Other researchers transformed cells with linked DNA including, for example: Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248, 247 (1980); Mantei *et al.*, "Rabbit  $\beta$ -globin mRNA production in mouse L cells transformed with cloned rabbit  $\beta$ -globin chromosomal DNA," *Nature* 281(5726):40-46 (1979). Furthermore, Wigler *et al.* later credited Dr. Weissman's group with reporting linked transformation in 1979. Perucho and Wigler, "Linkage and Expression of Foreign DNA in Cultured Animal Cells," *Cold Spring Harbor Symposia on Quantitative Biology* 1981. 45:829-838, 829. Therefore, one of ordinary skill in the art as of February 25, 1980 would have recognized that cloning vectors for use in transforming cells included a DNA I linked to a selectable marker.

66. In addition, those of ordinary skill in the art as of February 25, 1980 would have commonly been linking DNA I and a DNA encoding a selectable marker. For example, the insertion of DNA I and a DNA encoding a selectable marker into plasmid or phage vectors for the purpose of generating large amounts of DNA for transformation or other experiments was a common procedure. See the discussion below in section 3(e)(i)(2)(a); see also Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248 (1/1980); Weissmann *et al.*, "Expression of cloned viral and chromosomal plasmid-linked DNA in cognate host cells," in *From Gene to Protein: Information Transfer in Normal and Abnormal Cells* (Russell *et al.* eds.) Academic Press, Inc., pgs. 99-132, 99 (1979); Weissmann, "Future Trends: Reversed genetics," *TIBS* N109-N111, N110 (1978).

67. Thus, as of February 25, 1980, the "construct" comprising DNA I linked to DNA II in claim 1 of the '275 patent was obvious from claim 2 of the '017 patent.

- b) Both claims recite transformed CHO cells containing DNA I stably incorporated into chromosomal DNA, and DNA II, and the "construct" stably incorporated in claim 1 would be obvious in view of claim 2 of '017 patent**

68. Stable incorporation of a construct comprising DNA I linked to DNA II would have been obvious in view of claim 2/1 of the '017 patent, which recites "amplified foreign DNA I ... stably incorporated into the chromosomal DNA ... and ... amplified DNA II." As discussed above in Section 3(a)(iv)(2)(a), one of ordinary skill in the art as of February 25, 1980, would have thought it obvious that the DNA I and the DNA II encoding a dominant selectable phenotype in claim 2 of the '017 patent were linked. Furthermore, as discussed above in Section 3(a)(i)(2)(b),

104. Additionally, one of ordinary skill in the art would have known as of February 25, 1980 that the step selection culturing process which yields amplified DNA, such as that of claim 55, was developed using cells containing a *dhfr* gene as a model. Dolnick *et al.*, "Correlation of Dihydrofolate Reductase Elevation with Gene Amplification in a Homogeneously Staining Chromosomal Region in L5178Y Cells," *J. Cell Bio.* 83:394-402 (1979); Gupta *et al.*, "Purification and Properties of Dihydrofolate Reductase from Methotrexate-Sensitive and Methotrexate-Resistant Chinese Hamster Ovary Cells," *Can. J. Biochem.* 55(4):445-52 (1977); Nunberg, *et al.*, "Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line," *Proc. Natl. Acad. Sci., USA* 75(11):5553-5556 (1978); Alt *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370, 1357 (1978); Schimke *et al.*, "Amplification of dihydrofolate reductase genes in methotrexate-resistant cultured mouse cells," *Cold Spring Harbor Symp. Quant. Biol.* XLII:649-657, 649 (1978); Biedler and Spengler, "Metaphase chromosome anomaly: Association with drug resistance and cell-specific products," *Science* 191:185-187 (1976); Schimke *et al.*, "Gene Amplification and Drug Resistance in Cultured Murine Cells," *Science* 202:1051-1055, 1054 (1978).

105. In addition, *dhfr* genes were used in transformation experiments prior to February 25, 1980. Examples of these uses and other transformation experiments prior to February 25, 1980 were summarized in a 1980 publication by Wright *et al.*: "Other genes recently transferred with isolated chromosomes include those for dihydrofolate reductase (DHFR) (Sekiguchi *et al.*, 1975; Lewis *et al.*, 1980; Srinivasan and Lewis, 1980), various viral sequences (Ebina *et al.*, 1974; Shani *et al.*, 1976; Sabourin and Davidson, 1979; Scangos *et al.*, 1979) and the growth transformation phenotype (Cassingena *et al.*, 1978)." Wright *et al.*, "Somatic Cell Genetics: A Review of Drug Resistance, Lectin Resistance and Gene Transfer in Mammalian Cells in Culture," *Canadian Journal of Genetics and Cytology* 22(4):443-496, 474 (1980). For example, Dr. Lewis used a *dhfr* as a selectable marker to transform MTX-sensitive CHO cells, and presented that work at a meeting in May 1979, the proceedings of which were published in March 1980. Srinivasan and Lewis, "Transfer of the Dihydrofolate Reductase Gene Into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," in *Introduction of Macromolecules into Viable Mammalian Cells*, A Wistar Symposium Workshop Held at SugarLoaf Center Philadelphia, Pennsylvania, (May 2-4, 1979) (Baserga *et al.* eds.) Alan R. Liss, Inc., pg. 27-45, 43 (1980); Lewis *et al.*, "Parameters Governing the Transfer of the Genes for Thymidine Kinase and Dihydrofolate Reductase into Mouse Cells Using Metaphase Chromosomes or DNA," *Somatic Cell Genetics* 6(3):333-348, 346-347 (1980).

106. In addition, scientists also suggested using a *dhfr* as a selectable marker in a vector to transform eukaryotic cells. Chang *et al.*, "Phenotypic Expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase," *Nature* 275:617-624, 623 (1978); Chasin, Abstract of Grant Number 2R01GM022629-04, "Mitotic Recombination in Cultured Mammalian Cells" awarded to Lawrence A. Chasin for fiscal year 1978 ("Finally, mutants deficient in dihydrofolate reductase activity will be sought, for eventual use in DNA mediated transformation experiments using cloned genes."); Chasin and Urlaub, "Selection of Recessive Mutants at Diploid Loci by Titration of the Gene Product: CHO Cell Mutants Deficient in Dihydrofolate Reductase Activity," in *Banbury Report 2 Mammalian Cell Mutagenesis: The Maturation of Test Systems* (Hsie *et al.* eds.) Cold Spring Harbor Laboratory, pg. 201-209, 201, 208 (1979).

473, 471 (1978); Loh and Gainer, "The Role of the Carbohydrate in the Stabilization, Processing, and Packaging of the Glycosylated Adrenocorticotropin-Endorphin Common Precursor in Toad Pituitaries," *Endocrinology* 105:474-486, 485 (1979). Because CHO cells were known to glycosylate foreign proteins, a DNA encoding a glycoprotein would have been an obvious gene of interest to use to transform CHO cells as of February 25, 1980.

183. Furthermore, as of February 25, 1980, mammalian cells had been transformed with a DNA I encoding a glycoprotein linked to a selectable marker. Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248, 247 (1/1980); *see also* Taniguchi *et al.*, "Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," *Proc. Japan. Acad.* 55 Ser. B:464-469, 464 (1979) ("Interferons are species specific glycoproteins . . . cloned DNA may serve as a potent interferon producer in bacterial or in cognate host."). It would therefore have been further obvious to one of ordinary skill in the art as of February 25, 1980 that the gene of interest in the transformed cell of claim 4 of the '017 patent could be a DNA encoding a glycoprotein.

184. Therefore, to one of ordinary skill in the art as of February 25, 1980, the DNA I encoding a "glycoprotein" in claim 4 of the '275 patent would have been obvious in view of the DNA I corresponding to a gene of interest in claim 4 of the '017 patent.

185. The comparison of the two claims is further represented in Appendix A, Figure 4-4.

186. Thus, when comparing claim 4 of the '275 patent to claim 4 of the '017 patent, claim 4 of the '275 patent is obvious.

**(vi) Claim 4 of the '275 patent is obvious in view of claim 5 of the '017 patent**

187. Claim 4 of the '275 patent is dependent upon claim 3 of the '275 patent, while claim 5 of the '017 patent is dependent upon claim 1 of the '017 patent. Thus, the comparison of claim 4 of the '275 patent to claim 5 of the '017 patent is very similar to the analysis in my comparison of claim 3 of the '275 patent to claim 2 of the '017 patent. Claim 5 and claim 2 of the '017 patent are very similar except that claim 2 specifies that the DNA I "encodes a proteinaceous material which is not associated with a selectable phenotype," while claim 5 concerns a method of obtaining the "proteinaceous protein" encoded by DNA I. Accordingly, I incorporate the analysis in the comparison of claim 1 of the '275 patent to claim 2 of the '017 patent, as set forth in Section 3(a)(iv).

**(2) The other elements in claim 5 of the '275 patent are obvious in view of claim 55 of the '216 patent**

196. As discussed above in Section 3(a)(i)(2)(a), the transformed CHO cell in claim 5 of the '275 patent would have been obvious to one of ordinary skill in the art as of February 25, 1980 in view of the transformed eucaryotic cell in claim 55 of the '216 patent.

197. In addition, as I discussed above in Section 3(a)(i)(2)(b), it would have been obvious to one of ordinary skill in the art as of February 25, 1980, that the process of claim 55/54 will result in both DNA I and DNA II stably incorporated into the chromosomal DNA of the transformed cell.

**a) "DNA I or DNA II or both being attached to bacterial plasmid DNA or phage DNA" in claim 5 of the '275 patent would have been obvious**

198. The "DNA I or DNA II or both being attached to bacterial plasmid DNA or phage DNA" in claim 5 of the '275 patent would have been obvious to one of ordinary skill in the art as of February 25, 1980 in view of the molecule formed by linking DNA I and DNA II in claim 55 of the '216 patent. As of February 25, 1980, I would have understood the term phage, or bacteriophage to mean a virus which infects bacteria and a plasmid to mean any extrachromosomal self-replicating element of a cell. These definitions are based on my experience and understanding and are consistent with what is described in the Axel and '275 patents. Thus, "plasmid DNA" or "phage DNA" is any DNA that is derived from a phage or a plasmid.

199. It was known to those of ordinary skill in the art by February 1980 to insert DNA I and/or DNA II into plasmid or phage vectors for transforming eucaryotic cells. *See, e.g.*, Weissmann, "Future Trends: Reversed genetics," *TIBS* N109-N111, N109 (1978); Furthermore, Chang *et al.* also suggested the use of the *dhfr* gene which that group cloned as a eukaryotic transformation vector: "As the cloned coding sequence for mouse DHFR is selectable in higher organisms as well as in bacteria, it constitutes a powerful tool for the construction of eukaryotic cloning vectors." Chang *et al.*, "Phenotypic Expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase," *Nature* 275:617-624, 617, 623 (1978) (Reported cloning of *dhfr* in a plasmid).

200. In addition, researchers had transformed eucaryotic cells with DNA I and/or a selectable marker linked to plasmid and/or phage DNA prior to February 1980. *See, e.g.*, Weissmann *et al.*, "Expression of cloned viral and chromosomal plasmid-linked DNA in cognate host cells," in *From Gene to Protein Information: Transfer in Normal and Abnormal Cells*, Proceedings of the Miami Winter Symposia (held 1/1979) (Russell *et al.* eds.) Academic Press, Inc., pgs. 99-132 (1979); Mantei *et al.*, "Rabbit  $\beta$ -globin mRNA production in mouse L cells transformed with cloned rabbit  $\beta$ -globin chromosomal DNA," *Nature* 281(5726):40-46 (1979); Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248 (1/1980); Hamer and Leder, "Expression of the chromosomal mouse  $\beta^{maj}$ -globin gene cloned in SV40," *Nature* 281:35-40, 40 (1979); Wigler *et al.*, "Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total Cellular DNA as Donor," *Cell* 14:725-731, 730 (1978) ("An alternate approach [to transformation with

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**REBUTTAL EXPERT REPORT OF BRUCE J. DOLNICK**

understood.” Ruddle Report at pp. 26-27). Although the mechanism of amplification was not completely understood as of February 25, 1980, that incompleteness of understanding would not make stable incorporation of DNA II non-obvious, or provide any distinction between the ‘275 patent claims and prior patent claims. None of the ‘275 patent claims 3-19 specify any particular mechanism of forming stably incorporated DNA II or amplified DNA I and amplified DNA II.

6. Dr. Ruddle’s discussion of what was not known about the mechanism of amplification ignores that the relevant aspects of amplification were sufficiently understood to make obvious stably incorporated DNA II, and also mischaracterizes the state of knowledge regarding the mechanism of gene amplification known to those of ordinary skill in the art as of February 25, 1980. First, Dr. Ruddle inaccurately characterizes the state of the art in February 1980 by incorrectly suggesting that an article describing a potential theory for the mechanism of gene amplification, which later turned out to be incorrect, was published in February 1980:

In February 1980, Schimke postulated that gene amplification might have occurred as a consequence of reverse transcription of mRNA into DNA. In reverse transcription, it would have been theorized that mRNA is turned back into a form of DNA. If this was in fact the mechanism of amplification—and in February of 1980 there would be no way of discounting it—then one would assume that two pieces of DNA linked together either before insertion into a cell or just after insertion into a cell would not stay linked together following amplification.

Ruddle Report at pp. 27-28. However, the reference by Schimke *et al.* cited by Dr. Ruddle, was published in 1978, not 1980, and was the first paper on amplification from Dr. Schimke’s lab. See Alt *et al.*, “Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells,” *J. Biol. Chem.* 253(5):1357-1370 (1978). In addition, Dr. Schimke’s article discussed other theoretical mechanisms of amplification. More importantly, by February 25, 1980 (two years later), much more was understood about amplification and the mechanisms of amplification, and the theory that reverse transcription of mRNA was involved in gene amplification was known to be invalid.

7. Furthermore, as of February 25, 1980, one of ordinary skill in the art would have expected multiple copies (“amplified”) of DNA II in transformed cells grown under selective conditions, as in Claim 54 of the ‘216 patent, to be stably incorporated. For example, Dr. Schimke’s group reported in 1978 that amplified copies of the *dhfr* gene in CHO cells were located in a homogeneously staining region of the chromosome. Nunberg, *et al.*, “Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line,” *Proc. Natl. Acad. Sci., USA* 75(11):5553-5556 (1978). This is also consistent with Dr. Ruddle’s observation that “[t]he vast majority of DNA in a normal cell is associated with the chromosomes.” Ruddle Report at p. 23. In addition, as I noted in my initial report, in my experience I never observed unstable incorporation of amplified *dhfr* genes during the development of gene amplified cell lines. Dolnick Report at ¶ 31.

8. Therefore, stable incorporation would have been the obvious, expected outcome of transformation as a result of selection for the dominant selectable phenotype encoded by DNA II

recited in the claims of the prior Axel patents. Dolnick Report at ¶¶ 28-30. As further discussed in my initial report, one of skill in the art reading prior patent claims would have understood that DNA II is used as the selectable marker to select or identify cells which have undergone transformation, and that under selective conditions, DNA II would be expected to be stably incorporated into chromosomal DNA. Dolnick Report at ¶¶ 29-30. It was also known to those of ordinary skill in the art as of February 25, 1980, that *dhfr* could be used as a selectable marker, and that under increasing selective pressure, transformants with increased numbers of (i.e., amplified) *dhfr* genes would be identifiable. In addition, it was known that amplified *dhfr* genes were amplified as part of a very large piece of DNA referred to as an amplicon. Furthermore, it was known that amplicons are very large compared to the size of any one gene, and in fact were known to be large enough to contain many genes. See, e.g., Nunberg *et al.*, "Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line," *Proc. Natl. Acad. Sci., USA* 75(11):5553-5556 (1978); Dolnick *et al.*, "Correlation of Dihydrofolate Reductase Elevation with Gene Amplification in a Homogeneously Staining Chromosomal Region in L5178Y Cells," *J. Cell Bio.* 83:394-402 (1979). Furthermore, it was obvious to persons of ordinary skill in the art that the DNA I stably incorporated into the chromosomal DNA of the transformed cell would be linked to the DNA II, meaning that they would be in close proximity to each other within the chromosomal DNA. Thus, it was obvious that DNA I and II which are in close proximity to each other would exist in an amplicon and be co-amplified and thus stably incorporated.

9. Dr. Ruddle also states that: "It is not the case that DNA II is necessarily incorporated into the chromosome merely because DNA II and DNA I are amplified and DNA I is stably incorporated into the chromosome. In fact, scientific observations both in February 1980 and subsequently establish that amplified DNA II can reside within extra-chromosomal elements such as DMs." (Ruddle Report at pp. 25-26). However, it would have been obvious to one of ordinary skill in the art as of February 25, 1980 that cells with amplified, stably incorporated DNA I could only be obtained through co-amplification with stably incorporated DNA II in any practical manner.

10. Dr. Ruddle's drawing a distinction between amplified genes in double minute chromosomes and stably incorporated amplified genes therefore does not establish that stably incorporated DNA II was not obvious. As of February 1980, enough was known regarding unstable inheritance of double minute chromosomes to make it obvious to select and obtain transformed cells containing stably incorporated DNA II. For example, by 1977, Levan *et al.* had published that double minute "chromosomes" were inherited in a non-Mendelian fashion and eliminated when cells were cultured *in vitro*. Levan *et al.*, "Double Minute Chromosomes are Not Centromeric Regions of the Host Chromosomes," *Hereditas* 83:83-90 (1976); Levan *et al.*, "Experimental Elimination and Recovery of Double Minute Chromosomes in Malignant Cell Populations," *Hereditas*, 86:75-90, 84 (1977); see also Kaufman *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci U.S.A.* 76 (11):5669-5673 (1979). It was therefore known to those of ordinary skill in the art from at least 1976 that double minute chromosomes would not be stably inherited, and those of skill in the art as of February 25, 1980 knew how to determine whether or not DNA was stably incorporated into the chromosome. Thus, when cells are transformed and cultured, for example, under suitable conditions or conditions necessary to result in "amplified" DNA or production of proteinaceous material (requirements of the '216 and '017

patents), it would be obvious to one of ordinary skill in the art that DNA I and DNA II are both stably incorporated into the chromosomal DNA of the transformed cell.

11. If both DNA I and DNA II are linked and DNA I is stably incorporated, it would have been obvious that DNA II would also be stably incorporated since it codes for the dominant selectable phenotype. Dolnick Report at ¶¶ 28-31. Transformation using a DNA I linked to a selectable marker (such as a DNA II) had been suggested by 1978 and was demonstrated prior to February 25, 1980. Weissmann, "Reversed Genetics," *TIBS* N109-N111 (1978); Weissmann *et al.*, "Expression of Cloned Viral and Chromosomal Plasmid-Linked DNA" in *From Gene to Protein: Information Transfer in Normal and Abnormal Cells* (Russell *et al.* eds.) Academic Press, Inc., pp. 99-132 (1979). Prior to 1980, persons of ordinary skill in the art had transformed cells with linked DNA. Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. U.S.A.* 77(1):244-248 (1980); Mantei *et al.*, "Rabbit  $\beta$ -globin mRNA production in mouse L cells transformed with cloned rabbit  $\beta$ -globin chromosomal DNA," *Nature* 281(5726):40-46 (1979). In addition, stable incorporation of a selectable marker following transformation was known to those of skill in the art as of February 25, 1980. *E.g.*, Pellicer *et al.*, "The Transfer and Stable Integration of the HSV Thymidine Kinase Gene into Mouse Cells," *Cell* 14:133-141, 133 (1978) ("Biochemical transformation involves the stable acquisition of a new trait which can be readily detected by the ability of cells to grow under appropriate selective conditions. . . . [A]ll transformed clones examined contain . . . the *tk* gene [] covalently integrated into the DNA of all transformants . . . . These results demonstrate the stable integration of HSV DNA into mammalian cells."); Dolnick Report at ¶ 30.

12. At page 27 of his report, Dr. Ruddle continues to focus on knowledge in the art which is not relevant to whether stably incorporated DNA II would be obvious. "There was not sufficient experimental evidence or guidance in the field to allow scientists at any level of skill to have confidence about what would happen when and if two pieces of linked foreign DNA were amplified, even if one knew (as in claim 71 of the '216 patent) that DNA I is incorporated into the chromosomal DNA." This appears to ignore whether what was known as of February 25, 1980, in view of a corresponding claim in a prior Axel patent, would have made obvious stably incorporated DNA II in a claim of the '275 patent. For example, if it were obvious that amplified DNA I and amplified DNA II were linked, and DNA I were stably incorporated, as in claims of the '017 patent, it would have been obvious to those of ordinary skill in the art that DNA II would also be stably incorporated even if the mechanism of amplification were not known. Alternatively, if DNA I and II were linked, and then amplified (as in claim 54 of the '216 patent), it would have been obvious that DNA I and DNA II would be stably incorporated and co-amplified.

**(b) Production of a "Glycoprotein of Interest" Does Not Render Claim 19 of the '275 Patent Non-Obvious**

13. I disagree with Dr. Ruddle's statements that "a person of ordinary skill in the art (or for that matter a person of extraordinary skill), with knowledge of the original Axel claims, would not have considered it obvious to construct a eukaryotic cell (and in particular a mammalian or Chinese Hamster Ovary cell) with the characteristics of the host cell of claim 19 of the '275 patent that was competent to produce the 'glycoprotein of interest' of claim 19, and culture it under appropriate conditions to obtain that glycoprotein." Ruddle Report at p. 9.

glycoprotein linked to a selectable marker. Lai *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl Acad. Sci. U.S.A.* 77(1):244-248, 247 (1/1980). See also Taniguchi *et al.*, "Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," *Proc. Japan. Acad.* 55 Ser. B:464-469, 464 (1979) ("Interferons are species specific glycoproteins . . . cloned DNA may serve as a potent interferon producer in bacterial or in cognate host."). It would therefore have been further obvious to one of ordinary skill in the art as of February 25, 1980 that the gene of interest in the transformed cell of claim 4 of the '017 patent could be a DNA encoding a glycoprotein.

21. Furthermore, Dr. Ruddle argues that cultured cells can be very different from endogenous cells as they exist in the body and thus, "one could not have reasonably predicted in February 1980 whether the ability of a cell to perform a particular function would be retained, modified, or lost in the course of continuous propagation in culture." Ruddle Report at p. 12. Even if it were known as of February 25, 1980, that taking cells out of their natural environment and culturing them for long periods in the laboratory might alter certain functions needed for growth in culture, it was also well known that cultured cells produced functional proteins. As an example, cultured cells were commonly used to prepare and obtain enzymes, and the enzymes obtained from cultured cells were known to function faithfully compared to enzymes obtained from tissues in organisms. Those of skill in the art were further aware that it frequently is easier and more convenient to get proteins from cells in culture rather than from cells in living organisms; they also realized the basic biochemistry of the proteins produced by cells in culture was the same as for the proteins produced by cells in their natural environment. Therefore, the use of cultured cells to produce glycoproteins would frequently have been the method of choice and would have been obvious to one of ordinary skill in the art as of February 25, 1980.

22. I also do not agree with Dr. Ruddle that those of skill in the art as of February 25, 1980 would have been so concerned about the possibility of "squenching" of cellular machinery as a result of production of a glycoprotein encoded by foreign DNA I, that they would not have expected production of the glycoprotein. See Ruddle Report at p. 21. It is not obvious from Dr. Ruddle's statement that it was a subject of concern in the community as of February 25, 1980. For example, to support this point Dr. Ruddle only cites a single 1985 paper which he characterizes as "concluding that amplification did not impact processing." See Ruddle Report at p. 21 at n. 19. In addition, it was known by February 1980 that cells overproducing DHFR could be obtained in which 10% of the protein produced was DHFR instead of the 0.1% of protein in non overproducing cells. Furthermore, many glycoproteins are secreted. Therefore, glycoproteins produced in a transformed cell would not have been expected to remain in the cell, so that there would be fewer effects on the cell from accumulation of glycoproteins as a result of increased production. Additionally, claim 19 does not recite that any particular amount of glycoprotein needs to be produced.

23. With respect to comparisons of claim 19 to claim 3 of the '017 patent, Dr. Ruddle states that "Claims 1-4 do not report on whether the cell is competent to transcribe and translate DNA I." Ruddle Report at p. 18. However, as I discussed in my initial report, it would have been obvious to one of ordinary skill in the art as of February 25, 1980, that the transformed CHO cells in the claims of the '017 patent are competent to express protein and to glycosylate glycoproteins encoded by DNA I. As of February 25, 1980, it was known that CHO cells glycosylate proteins, even foreign proteins. Narasimhan *et al.*, "Control of Glycoprotein Synthesis: Lectin-Resistant